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Effects of Incubation Temperature and Relative Humidity on Embryonic Development in Eggs of Red-Winged Tinamou (*Rhynchotus rufescens*)

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Abstract: This study was conducted to assess the effects of incubation temperature (34°C, 36°C and 38°C) and relative humidity (RH, 50% and 60%) on egg weight loss, embryo mortality, hatchability, incubation time and chick weight in eggs from red-winged tinamou. The eggs were placed in incubators that were operated at 34°C, 36°C, or 38°C and 50% or 60% RH (mean wet bulb temperatures of 28°C and 30°C, respectively) from day 1 to hatching. Each treatment had two replicate groups of 30 eggs each. Hatchability varied with incubation temperature and RH and was highest for eggs incubated at 36°C and 60% RH and lowest for eggs incubated at 38°C. Early, intermediate and late embryo mortality were highest at 38°C, 38°C/50% RH, and 50% RH, respectively. Incubation period was longest at 34°C and shortest at 38°C/50% RH. Present results show the highest hatchability of red-winged tinamou eggs after incubation at 36°C and 60% RH; highest embryo sensitivity to high temperature in the early period of incubation (1 to 7 days), to high temperature and low RH in the second period of incubation (8-14 days) and to low RH in the late period of incubation (after 15 days) and shortest incubation period with increasing temperature and RH.

Key words: Aves, egg incubation, hatchability, mortality, tinamid

INTRODUCTION

The developmental environment is of crucial importance for oviparous species because it may affect embryogenesis, inducing detrimental phenotypic alterations if eggs are exposed to extreme variations in environmental conditions. Temperature and humidity are environmental factors that have a great influence on incubation time and overall egg water loss during incubation, with subsequent effects on embryonic viability and weight of chick at the time of hatching (Simkiss, 1980; Peebles *et al.*, 1987; Decuypere and Michels, 1992; Suarez *et al.*, 1997; Finkler *et al.*, 1998; Morita *et al.*, 2010).

Despite the considerable body of research devoted to egg incubation in poultry science (Bertran and Burger, 1981; Peebles and Brake, 1987; Swann and Brake, 1990a,b; Decuypere and Michels, 1992; Vick *et al.*, 1993; Satteneni and Satterlee, 1994; Deeming, 1996), the effects of temperature and humidity on egg development remain unknown for most wild bird species. Considering that the relationship between these two incubation criteria determines successful embryogenesis in eggs of domesticated birds, it is reasonable to expect that it also influences egg development of tinamids, which also are precocial birds. The tinamids comprise exclusively Neotropical palaeognathous birds, distributed from Northwest Mexico to southern South America (Cabot, 1992). These birds have poor flight capability and occupy diverse

environments, such as steppes and rain forests (Sick, 1985). Little scientific information is available concerning artificial incubation characteristics of tinamid eggs. In previous studies, we reported a negative correlation between incubation period and temperature (Nakage, 2003) and an incubation temperature ranging from 35.5°C to 36.5°C at 60% Relative Humidity (RH) could be used to incubate red-winged tinamou eggs (Nakage *et al.*, 2003). To our knowledge, the effects of temperature and RH on egg development in red-winged tinamou have not been examined previously. In this study, we examined the effects of incubation temperature and RH on egg weight loss, hatchability, embryonic mortality, incubation time, and chick weight at the time of hatching in red-winged tinamou.

MATERIALS AND METHODS

Eggs utilized in this study were obtained from red-winged tinamou, *Rhynchotus rufescens* (Tinamiformes, Tinamidae) maintained in the Wild Birds Sector of the Animal Science Department of the Faculty of Agricultural and Veterinary Sciences, São Paulo State University, under permit of the environmental authorities of the Brazilian Institute of Environmental and Renewable Natural Resources - Brazilian Ministry of Environment (IBAMA, process n° 1/35/92/0882-5). The experimental protocol used in the present study was approved by the Ethical Committee in Animal Use of the cited Faculty (protocol n° 001488/11).

The tinamous, *Rhynchotus rufescens*, were in their second breeding season and of 21 to 28 months of age. They were housed in a conventional masonry poultry house with a concrete floor and asbestos tiles. Birds were housed in 40 pens, containing one male and two females each, separated by a 40-cm wall and covered with 2.0 m x 1.0 m x 2.1 m wire mesh to isolate the experimental birds. Pens were equipped with tube feeders and bell drinkers and the floor was covered with coast-cross (*Cynodon dactylon*) hay litter. The birds received water and feed *ad libitum*. The diet (pelleted corn and soybean meal; containing 2,800 kcal ME/kg feed and 15% crude protein) was formulated based on the studies of Moro *et al.* (2002). During the experimental period, a lighting program of 18 hrs of light was applied daily.

Eggs were collected three times daily from birds housed in pens, during the 3-month period (October to January), corresponding to peak egg production. After collection, eggs were weighed and individually identified by box, day laid, and laying order. The eggs were immediately stored at 20°C and 65% RH; such storage conditions increase hatchability and neither affect egg weight nor chick weight at the time of hatching (Nakage, 2003). Mean weight loss of eggs during storage was approximately 0.5-1% for all treatment replicates, which was assumed to not be significant. Incubations were initiated after each collection. Therefore, eggs were fresh (i.e., 1-2 days old) when set. All eggs were individually reweighed before incubation. The eggs were placed in incubators (Premium Ecológica IP 70) with automatic controls of temperature [34°C, 36°C, or 38°C and 50% or 60% RH (mean wet bulb temperatures of 28°C and 30°C, respectively)] and egg turning (1 turning every 2 hrs). Each treatment had two replicate groups of 30 eggs.

The response variables considered in this study were egg weight loss, incubation time, hatchability and mortality of fertile eggs and weights at hatching. Egg weight loss was calculated as the percentage of egg weight before initiation of incubation: Eggs were weighted prior to the initiation of incubation and on day 18 of incubation. Incubation time to hatching was given in hours and days. Hatchability and embryonic mortality were expressed as the percentage of fertile eggs. All unhatched eggs were examined macroscopically for fertility and classified as early (0 to 7 days), intermediate (8 to 14 days), and late (from 15 days) embryonic mortality. Chick weight at hatching was determined using an electronic balance that was accurate to 0.01 g and expressed as the percentage of egg weight before incubation.

The data were analyzed utilizing temperature and RH as main effects. All statistical analyses were conducted using the GLM procedure of SAS software (SAS Institute, 2004) and statements of statistical significance based

on $p \leq 0.05$ unless otherwise stated. Pearson correlation coefficients were calculated to determine the relative importance of the temperature and RH in determining hatchability, embryonic mortality, egg weight loss, incubation time and chick weight, as well as the relative importance of egg weight loss on incubation time, mortality, hatchability and chick weight.

RESULTS

Hatchability and mortality: The overall effects of incubation temperature, incubation RH and interaction between incubation temperature and RH on Hatchability (HAT), total Embryonic Mortality and Early (EEM), Intermediate (IEM) and Late (LEM) embryonic mortality in fertile eggs are shown in Table 1. There were significant effects ($p < 0.05$) of the incubation RH on HAT, TEM and IEM, as well as of the incubation temperature and interaction between incubation temperature and RH on all analyzed parameters.

Table 1: Effects of incubation temperature (34°C, 36°C and 38°C) and relative humidity (50% and 60%) on Hatchability (HAT), Total Embryonic Mortality (TEM) and Early (EEM), Intermediate (IEM) and Late (LEM) embryonic mortality in fertile eggs of *Rhynchotus rufescens*

	HAT (%)	TEM (%)	EEM (%)	IEM (%)	LEM (%)
Temperature (T, °C)					
34	34.56	66.25	21.78	8.02	23.29
36	59.94	40.05	10.78	3.81	20.27
38	10.54	87.42	53.11	18.85	26.84
Relative Humidity (RH, %)					
50	28.96	71.03	27.97	14.10	37.04
60	48.33	51.67	26.73	4.33	21.30
Probability					
T	0.0002	0.0002	0.0014	0.0277	0.5207
RH	0.0429	0.0429	0.9197	0.0432	0.0029
TxRH	0.0005	0.0005	0.0287	0.0036	0.0236

A-B: indicate significant differences among means (columns) ($p \leq 0.05$)

According to the interaction between incubation temperature and RH (Fig. 1A), the influence of RH on HAT, expressed as the percentage of fertile eggs, was reported only for eggs incubated at 36°C; in such cases, HAT was higher at 60% than at 50% RH. The highest HAT occurred for eggs incubated at 36°C and 60% RH, and the lowest HAT for eggs incubated at 38°C independent of RH. When egg incubation occurred at 50% RH, there was no difference in HAT between eggs incubated at 34°C and 36°C, which presented higher HAT compared to eggs incubated at 38°C. When eggs were incubated at 60% RH, the highest HAT occurred for eggs incubated at 36°C and the lowest for eggs incubated at 38°C. HAT of fertile eggs presented a moderate negative correlation with incubation temperature ($r = -0.41$) and egg weight loss ($r = -0.31$), and a moderate positive correlation with RH ($r = 0.47$). The relationship between HAT and time of incubation

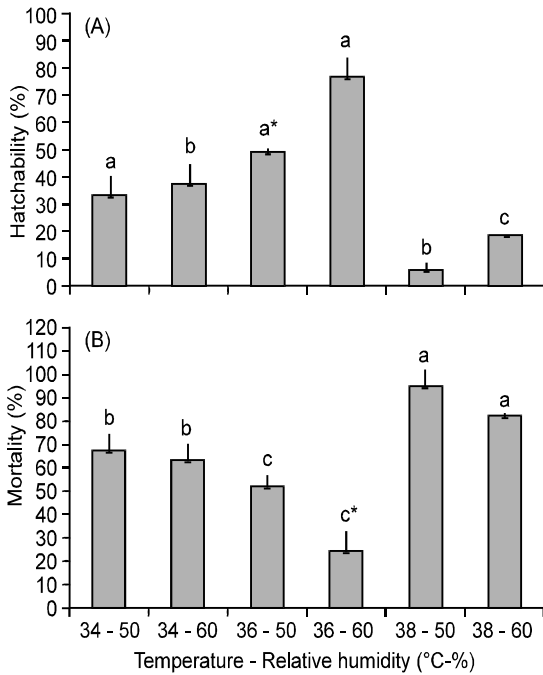


Fig. 1: Interaction between incubation temperature (°C) and relative humidity (%) on hatchability (A) and embryonic mortality (B) in fertile eggs of *Rhynchosotus rufescens*. Distinct letters indicate significant differences among temperatures within each relative humidity. *Significant difference between relative humidity at the same temperature ($p \leq 0.05$)

was not significant ($r = -0.005$). The effects of incubation temperature and RH on HAT were on the pattern of embryonic mortality. As shown in Figure 1B, there was an influence of RH on TEM only for eggs incubated at 36°C; in these cases, TEM was higher at 50% than at 60% RH. TEM was highest for eggs incubated at 38° independent of RH and lowest for eggs incubated at 36°C and 60% RH.

Figure 2 shows the influences of the interactions between incubation temperature and RH on EEM, IEM and LEM. There were significant differences in EEM between incubation RH in eggs incubated at 38°C; in these cases, EEM was higher at 50% than at 60% RH (Fig. 2A). The highest EEM occurred at 38°C and the lowest occurred at 36°C. Effects of incubation RH on IEM was reported in eggs incubated at 34°C and 38°C (Fig. 2B) and at both temperatures, IEM was higher at 50% RH than at 60% RH. All egg groups incubated at 60% RH had similar IEM. However, there were significant differences in IEM among temperatures for eggs incubated at 50% RH. IEM was higher for eggs incubated at 38°C than for eggs incubated at 34°C and 36°C; these latter incubations did not differ from one another. LEM was higher for eggs incubated at 50% than for eggs incubated at 60% RH (Fig. 2C). Additionally, no

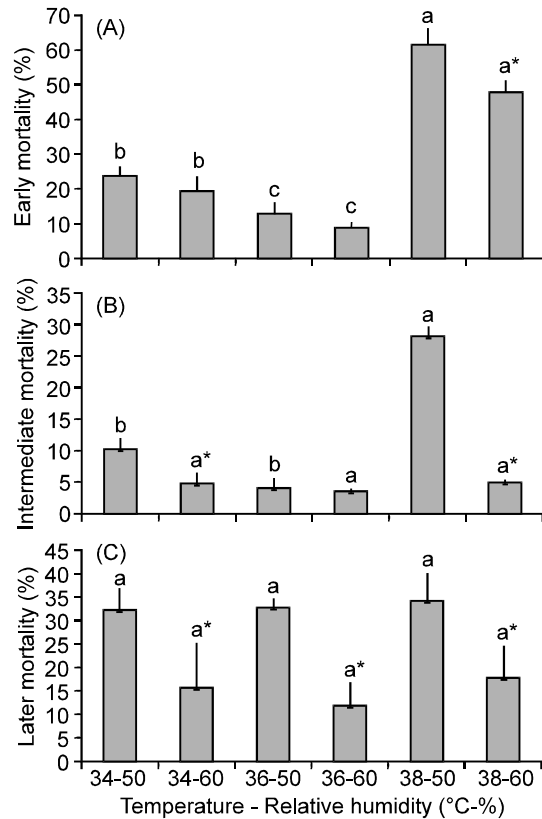


Fig. 2: Interaction between incubation temperature (°C) and relative humidity (%) on early (A), intermediate (B) and late (C) embryonic mortality in fertile eggs of *Rhynchosotus rufescens*. Distinct letters indicate significant differences among temperature within each relative humidity. *Significant difference between relative humidity at the same temperature ($p \leq 0.05$)

significant differences in LEM among incubation temperatures were found with respect to RH. EEM presented a moderate positive correlation with incubation temperature ($r = 0.55$) and a low positive correlation with egg weight loss ($r = 0.27$); the relationship between EEM and RH was not significant ($r = -0.03$). There was a moderate positive correlation between IEM and incubation temperature ($r = 0.42$), a low positive correlation between IEM and Egg Weight Loss (EWL) ($r = 0.22$) and a low negative correlation between IEM and RH ($r = 0.18$). LEM had moderate negative correlations with incubation temperature ($r = -0.41$) and RH ($r = -0.48$), but its relationship ($r = -0.05$) with EWL was insignificant.

Egg weight loss: EWL, given as a percentage of egg weight prior to incubation, was significantly influenced ($p < 0.05$) by incubation temperature, but not by incubation RH or by the interaction between incubation temperature

Table 2: Effects of incubation temperature (34°C, 36°C and 38°C) and relative humidity (50% and 60%; RH) on Egg Weight Loss (EWL) during incubation, Length of Incubation Period (LIP) and chick Body Weight (BW) at hatching (g, %) in *Rhynchotus rufescens*

	EWL (%)	LIP (days)	LIP (hours)	BW (g)	BW (%)
Temperature (T, °C)					
34	10.27B	24.54	591.20	37.88	69.34
36	11.96AB	21.82	506.67	38.48	65.79
38	14.37A	19.96	476.20	37.22	66.94
Relative Humidity (RH, %)					
50	13.08	22.15	528.89	37.63	68.91
60	11.09	21.40	516.71	38.10	69.71
Probability					
T	0.0098	0.0001	0.0001	0.3219	0.3202
RH	0.1511	0.5524	0.6787	0.6561	0.2526
TxRH	0.1534	0.0161	0.0033	0.8067	0.6230

A-B: indicate significant differences among means (columns) ($p \leq 0.05$)

and RH (Table 2). EWL during incubation was higher for eggs incubated at 38°C than for eggs incubated at 34°C. EWL had a moderate positive correlation with incubation temperature ($r = 0.57$) and moderate negative correlations with RH ($r = -0.39$) and incubation length given in hours and days ($r = -0.45$ and $r = -0.41$, respectively).

Length of incubation period: Length of Incubation Period (LIP) was significantly affected ($p < 0.05$) by incubation temperature and by the interaction between incubation temperature and RH (Table 2). As shown in Fig. 3, an influence of incubation RH on LIP was found only for eggs incubated at 38°C; in such cases, LIP was shorter at 60% than at 50% RH. Significant effects of temperatures on LIP were found at 50% and 60% RH. At 50% RH, LIP was longer at 34°C than at 36°C and 38°C, and these latter incubations did not differ from one another. At 60% RH, the LIP increased with increasing temperature. LIP, expressed in hours or days, had high negative correlations with incubation temperature ($r = -0.85$ and $r = -0.84$, respectively) and its correlations with RH ($r = -0.11$ and -0.16 , respectively) were insignificant.

Chick weight: As shown in Table 2, chick weight at hatching, expressed in grams or as the percentage of egg weight prior to incubation, was not influenced ($p > 0.05$) by incubation temperature, incubation RH, or the interaction between incubation temperature and RH. At hatching, chick weight was approximately 37.86 g and 67.37% of the egg weight prior to incubation. Although the influence of RH was not significant, when chick weight was analyzed for correlations with RH and EWL, moderate ($r = -0.32$) and low negative correlations ($r = -0.24$) were found, respectively.

DISCUSSION

The present study showed that HAT of red-winged tinamid fertile eggs can be influenced by incubation temperature, RH and EWL, as reported for other

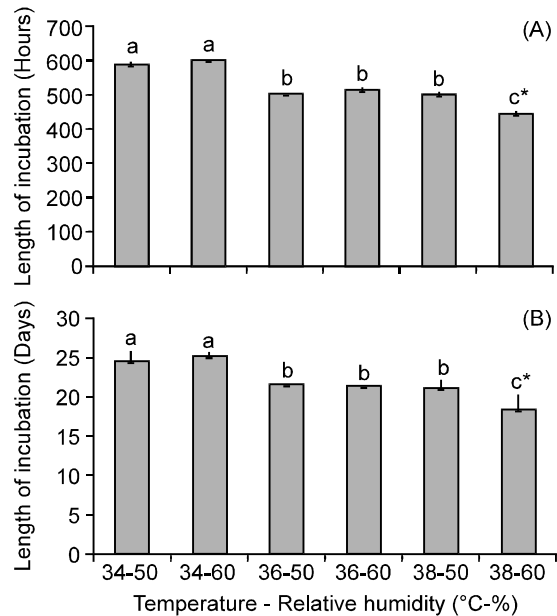


Fig. 3: Interaction between incubation temperature (°C) and relative humidity (%) on length of incubation period (A, hours; B, days) of *Rhynchotus rufescens* eggs. Distinct letters indicate significant differences among temperature within each relative humidity. *Significant difference between relative humidity at the same temperature ($p \leq 0.05$)

precocial avian, domestic fowl (Rahn *et al.*, 1979; Board, 1980; Wilson, 1991; Fassenko *et al.*, 1992; Vick *et al.*, 1993) and ostrich eggs (Gonzales *et al.*, 1999; Horbaczuk *et al.*, 1999), turkey and ducks (Christensen *et al.*, 2001; DuRant *et al.*, 2011). HAT was influenced by RH only when eggs were incubated at 36°C; HAT was markedly increased (76%) when eggs were incubated at 36°C/60% RH compared to eggs incubated at 34°C (35%) and 38°C (12%), likely due to lower embryonic mortality during the incubation period. These results support previous observations by Nakage *et al.* (2003) who analyzed the impact of incubation temperature (35°C, 36°C, 37°C, 38°C and 39°C) on the fertile eggs of red-winged tinamid incubated at 60% RH; they reported that HAT was higher at approximately 36°C.

EEM (1-7 days), IEM (8-14 days) and LEM (>15 days until hatching) were highest for eggs incubated at 38°C (~54.5%), 38°C-50% RH (~28%) and 50% RH (33%), respectively, indicating a phase-specific response of red-winged tinamids to temperature and RH during incubation. High incubation temperatures have greater effects on the early period of incubation, while low RH has greater effects on the late incubation period. For precocial birds, incubation periods can be divided into embryonic (1-7 days) and fetal (8 days to hatching) phases. Thus, our data demonstrated higher embryonic

sensitivity to high incubation temperatures and higher fetus sensitivity to low RH.

Mortality in eggs from domestic fowl has been related, among other things, to excessive or low egg water loss. Water loss above the optimal rate promotes embryonic death by dehydration, while very low water loss (<12%) causes embryonic hyper-hydration which makes difficult gas exchanges throughout eggshell membranes (Romanoff, 1930). Maudin (1993) established that 12-13% of egg weight loss up to transference at day 17 of incubation as an optimal value for domestic fowl eggs, with 11-14% as an acceptable value. Rosa *et al.* (2002) considered that HAT is optimized with 10.3% weight loss at an incubation temperature of 28.6°C. For red-winged tinamou (our results), the average egg weight loss ranged from 10.27% at 34°C to 14.37% at 38°C, with the highest HAT occurring with an average egg weight loss of 11.96% at 36°C. Our results differ from those obtained for rock partridge, *Alectoris graeca*, by Kirikci *et al.* (2004), who reported disproportionately reduced HAT in eggs that had lost less weight during incubation. During incubation, the rate of evaporative egg weight loss can be influenced by temperature and RH in the incubator, by eggshell thickness and by pore area and number. For optimization of domestic fowl egg incubation, Robertson (1961) and Tullett and Burton (1982) suggest utilizing 50% RH with a temperature of 37.5°C. Additionally, Vick *et al.* (1993) found that HAT was increased and EEM reduced by a wet bulb temperature of 28.3°C (50% RH) when compared with a wet bulb temperature of 30°C (58% RH) and a dry bulb temperature of 37.5°C. In the present study, the highest HAT for eggs of red-winged tinamou occurred at 60% RH and 36°C, incubation conditions in which the lowest overall embryonic mortality was reported. Effects of RH on EEM were observed only for eggs incubated at high temperatures (38°C), which were reduced approximately 22% by the addition of 50% RH. Additionally, low incubation RH (50%) increased the incidence of IEM in to 46% and 82% when egg incubation occurred at lower (35°C) and higher (38°C) temperatures, respectively and increased the incidence of LEM at 35°C, 36°C and 38°C (to 51%, 64% and 48%, respectively) compared with 60% RH. Thus, although no significant effects of RH have been reported on EWL, a significant negative correlation between EWL and RH was found, indicating that high water loss associated with low RH may increase both IEM and LEM.

The results from this study confirm that changes in incubation temperature and humidity can elicit changes in the incubation period of red-winged tinamou eggs as reported in the literature for other precocial avians. Low incubation temperatures extend the average incubation periods of red-winged tinamou by approximately 5 and 7 days compared to high temperatures at 50% RH (34°C: 24 days and 38°C: 21 days) and 60% RH (34°C:

25 days and 38°C: 18 days), respectively. These results support observations by Nakage *et al.* (2003) who reported an incubation period between 19 and 26 days at 36°C and 38°C, respectively, with 60% RH. Additionally, observations by Nogueira-Neto (1973) reported LIP of 19 to 21 days for red-winged tinamou. Our results indicate that incubation temperature is a primary determinant of embryonic developmental velocity in red-winged tinamou, similar to other precocial avians such as turkey (French, 1994), domestic fowl (Decuyper *et al.*, 1979) and ostrich (Hassan *et al.*, 2004).

The growth rate of the avian embryo is influenced by incubation conditions and determines the LIP (Ricklefs and Starck, 1998), which is a primary determinant of neonate size (Christensen *et al.*, 2002). Chick weight at hatching is also proportional to water loss during incubation (Simkiss, 1980; Finkler *et al.*, 1998). Although the LIP diminishes and the EWL increases with increasing incubation temperature, no significant relationship between chick weight and either temperature or LIP was reported in the current study. However, chick weight had moderate and low negative correlations with RH and EWL, respectively. Considering that fetal phases of incubation are characterized by great growth and weight gain, the relationship between chick weight and RH appears to be related to and influence on fetal phases.

In summary, this study showed that 1) incubations at 36°C/60% RH increase HAT in eggs of red-winged tinamou; 2) high temperature is highly damaging in the first phase of incubation, high temperature and low RH affect the second incubation phase and low RH affects the last phase and 3) incubation period becomes shorter at low temperatures.

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